SECTIONING FOR THE ELECTRON MICROSCOPE ACCOMPLISHED BY THE HIGH SPEED MICROTOME

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ELEVEN PLATES (TWELVE FIGURES)

During the few years since the electron microscope has become available as a scientific instrument, its great advantages as well as its disadvantages in comparison to the light microscope have unfolded to many observers. The amount of useful magnification which a microscope is capable of producing is limited by its power of resolution which is the instrument's ability to register fine detail. A light microscope is quite capable of a high degree of magnification, but the instrument's limit of resolution is reached around 1200–1500 magnification.[2] Further magnification above that limit by optical or photographical means will further enlarge the object or its picture, but will bring out no further detail. It is the fundamental endowment and outstanding advantage of the electron microscope to possess excellent power of resolution and sharp definition up to 20,000 diameters (Zworykin and Hillier, '44). Micrographs of such magnification, by virtue of their sharp definition, can very advantageously be further enlarged photographically up to 100,000 diameters.

Another great advantage of the electron microscope is its much greater depth of field. It presents the invaluable possibility of stereoscopic micrographs possessing such good con-

[1] The authors wish to express their full appreciation for the support of this work by a specific grant from the Lillia Babbitt Hyde Foundation.

[2] Using ultra violet illumination, resolution is increased to about 2,000 diameters of useful magnification.

trast as to enable the observer to study in one picture the entire depth of a specimen in its spatial, three dimensional arrangement (figs. 10 to 12).

The electron microscope, on the other hand, imposes limitations. The most serious handicap is the necessity that specimens must be dehydrated before they can be exposed to the high vacuum which must prevail all through the instrument for the passage of the electron beam. Another limitation is imposed by the low penetrating power of an electron beam. Because of this, most objects present a much higher opacity in the electron microscope than in the light microscope. The reason is the strong electron absorption. This is in proportion to the general mass of the object,[3] while light undergoes an absorption much less severe which in many instances occurs only in some specific wave lengths. Consequently even at comparatively high electron velocity the specimens must be small enough at least in one of their three dimensions — the dimension parallel to the beam — to reduce electron absorption as much as possible and allow sufficient penetration by interposing as little mass as possible in the path of the beam. Thereby detail becomes accessible and so can be revealed by electron microscopic examination. The most careful and elaborate techniques known for specimen preparation heretofore have not been able to overcome this difficulty of limited penetration if the natural size level of at least one dimension of the object was above a certain maximum. Fortunately the limitation was not due to the electron microscope instrument itself. It consisted rather of a lack of techniques to prepare sections of tissue sufficiently thin to meet the specific small dimensional requirements of the low penetrating power of the electron microscope. Of course, there are many materials which by their natural dimensions are suited for electron microscopic study. They are materials like fillers, clays, pigments, bacteria, viruses, colloids, etc. Excluded, however, were the highly important fields where the materials in their natural

[3] In crystalline objects the orientation of particles also controls electron absorption.

state were above the size limitations and could not be examined by the new instrument without first developing new techniques for tissue preparation. The internal structure of materials remained almost excluded from exploration by the new instrument.

The extensive study of structures by the light microscope employing sections sliced by hand or by microtomes may be considered one of the most important and fruitful fields explored by microscopy. Yet sections prepared by such known methods were far too opaque in the electron microscope and produced only coarse silhouettes. The commonly used electron velocity of the beam, accelerated by a potential of 60,000 volts, comes perhaps near the limits of practical electron microscope construction. This beam is not able to penetrate these sections. They must be thinner, by a factor of almost 10, to allow sufficient electrons to go through them so that satisfactory micrographs can be obtained. No practical method was known to produce such thin sections.

PRESENTLY KNOWN TECHNIQUES FOR SPECIMEN PREPARATION

In the absence of suitable sectioning methods, cytologists have begun the electron microscopic study of the morphology of tissue cells by two new techniques requiring no slicing. In one of these techniques the tissue components may be examined through the use of a segregation procedure by which the cells of a tissue sample are broken open and some of the components separated by centrifugation from a suspension (Claude and Fullam, '45). Suspended fractions may then be mounted separately on film-covered specimen screens. The other cytological technique of studying cell structure by the electron microscope was carried out by growing exceedingly thin cells in tissue cultures (Porter, Claude and Fullam, '45). This they accomplished by culturing living tissue fragments in a nutrient material on a film-covered glass slide under carefully controlled conditions. After fixation and washing, the film with a thin-grown cell on it was transferred to a specimen

screen and was found suitable for electron microscopic investigation. It revealed fine detail in the thinner cytoplasmic parts of the cell but the nucleus was too thick and dense to show structural detail.

The existing methods and particularly the microtomes for making sections for the light microscope produce sections approximately ten times too thick for the requirements of the electron microscope and they are thereby quite inadequate. Consequently it became of specific importance to develop an entirely new type of microtome and the associated techniques by which sections of many different types of biological materials could be produced in the necessary thinness and without deformation.

At first the only way to secure sections thin enough for the electron microscope seemed to be by the wedge method and theoretically it appeared feasible to cut wedges of tissue by hand and with an angle small enough so that the limited thin area of the wedge would permit sufficient electron penetration. A few publications appeared but the difficulty involved can best be estimated from the paper by Richards and Anderson ('42) who had to produce a thousand sections by hand to find one that was suitable. In addition they found it necessary to increase the velocity of their electron beam to 200,000 volts in order to obtain sufficient electron penetration. They displayed micrographs of insect cuticle as illustrations of their method.

The first and really important advance in the difficult subject came in November, 1943, by O'Brien and McKinley. They described in a short article a microtome whose cutting knife was carried by a fast revolving knife arm rotating at a speed of 12,500 RPM to produce a cutting speed of 140 feet per second. No results, however, were described or shown by them until a year later at the November, 1944, meeting of the Electron Microscope Society when they exhibited some micrographs which indicated that they had actually produced sections thin enough for electron penetration.

It may be accepted today that a very valuable contribution was made in their suggestion of high speed slicing and a theory of relationship between speed and angle of cutting and the strains produced in the specimen ahead of the knife would appear to be tenable. The higher the knife speed, the smaller the distribution or spreading of strain in the specimen ahead of the knife edge. The effect, it can be reasoned, is a two-fold one, namely a sharp separation of each single section from the tissue block and a minimum deformation within the section.

The knife of an ordinary microtome appears to remove tissue sections by the process of plastic flow at the knife edge. But a tissue block, hit by the high velocity impact concentrated in the sharp edge of the knife of a high speed microtome, possesses, in relation to the knife, such inertia that it acts like a brittle solid with the consequence that the sections appear to be chipped off. Sections so produced need not be larger than about 50 microns square.

SECTIONING WITH THE HIGH SPEED MICROTOME

The theory indicates that the thickness of a section that can be made is inversely proportional to the cutting speed. Therefore a series of high speed microtomes was constructed by us in which the cutting knife was fastened to the periphery of a high velocity wheel. By increasing rotational speeds and knife drum diameters, increasing cutting speeds were obtained (figs. 1, 2, 3). In an accompanying paper filed for publication in The Review of Scientific Instruments,[4] the authors have described in detail two modifications of the microtome, one of which is capable of knife speeds up to 1100 feet per second at 57,000 RPM of the knife bearing wheel.

The prepared tissue is fed into the path of the knife by a motor driven micrometer feeding mechanism, the rate of feed determining the thickness of the sections for a given speed of the cutting wheel. At top cutting speed almost a thousand sections per second are produced. Their thinness

[4] The Review of Scientific Instruments, vol. 17, no. 1, pp. 23–25, January, 1946.

may be reduced to 0.1 micron, though sections up to 0.3 or even 0.5 microns allow sufficient penetration with some tissues (figs. 11 and 12). For stereoscopic pictures, which are generally taken at a lower magnification, it is disadvantageous to slice thinner than 0.3 microns, since not sufficient depth may be left and structures may be cut away which could otherwise be seen in their spatial arrangement throughout the depth of a thicker section. On account of air turbulence and the microscopical size of these sections as well as their static charge, the collection of them becomes a difficult problem and a number of different methods for the collection of these sections are described in the above mentioned paper.

A difficult problem which particularly pertains to tissue sections is that they, on the average, contain from 75–90% water. The removal of this tissue water, required by the vacuum of the electron microscope, must be accomplished without distortion of the specimen and without creating artifacts in it. The remaining solids which are only a fraction of the original tissue weight must be preserved in full detail. At best, the connecting structures or filaments can not be expected to leave more than a fine network supporting the granular or other distinctly formed parts of the cell. The removal of any percentage of the tissue water by a method other than the most careful extraction of water-soluble solvents or by sublimation from non-crystalline ice, produced by low temperature freezing, would lead to serious contraction of the remaining structure. However, it has not been possible so far to successfully section a block of frozen-dried unembedded tissue. It invariably disintegrates into small fragments under the knife. Embedding after freeze-drying seemed to be equally unsatisfactory since marked distortion occurred in all cases.

A good deal of emphasis so far has been given to the production of sections thin enough for the electron microscope. It must appear clear, however, that high speed microtome sections are equally suitable for the light microscope since the

high speed microtome may be set to cut sections in any desired thickness to suit the requirements of either of the two types of microscopes.

FIXATION AND EMBEDDING

We have found that the exceedingly intricate detail revealed by the high resolution and sharp definition of the electron microscope is so delicate not only in itself but also in its spatial arrangement that a process of some hardening or solidification of the tissue must precede slicing. In addition, in order to gain sufficient support for the fixed tissue to withstand the impact of the knife and the necessary treatment of the sections after being cut, which is relatively rough for such fragile preparations, embedding before slicing is necessary. Materials such as bone and cartilage which have sufficient rigidity to support themselves under the knife impact may not require embedding. The common methods of fixation of tissue before sectioning for electron microscopy have produced only very limited results so far. Most of the fixatives used in light microscope techniques we found to cause distortion by contracting the fine structure and network that is left after removal of all water (figs. 9 and 10). The least distorted specimens we have obtained so far in the fixation of tissue were secured by treatment with osmium tetroxide before they were embedded (figs. 5, 6, 7, 8, 11 and 12).

Embedding in paraffin and similar waxes by the standard method allows excellent sectioning, but the waxes must be completely removed before the sections are suitable for electron microscope examination. Yet the extraction of the waxes produces, in many cases, distortion within the fragile sections by the surface forces which result from the evaporation of the extracting solvents. Also some of the lipids and fatty constituents of the tissue are removed together with the waxes. In order to reduce the chances of such distortion we considered it a matter of considerable importance to recognize that the high speed microtome can successfully slice materials far more brittle and harder than waxes. In consequence we were able

to select embedding materials from classes of materials which are able to volatilize from their solid state by sublimation and which in addition possess a melting point as low as paraffin, so that sensitive tissue can be embedded in them with no greater harm than in paraffin. One of the preferred materials we have developed for this purpose is an eutectic mixture of camphor and naphthalene, which has good cutting properties and which melts at 32°C. Our experiments with this new embedding technique, which is described in detail in our accompanying paper in The Review of Scientific Instruments,[5] have indicated that such sublimable materials give sufficient support to the specimen for cutting as well as for collecting the sections. After their removal by volatilization, which can best be carried out by exposure to moderate vacuum for a short period, the sections are free of them as far as the electron microscope can detect (fig. 12).

In great contrast to embedding techniques for the ordinary microtomes, ice may be used as a supporting and embedding substance in high speed microtome sectioning. It is well known that ice, if it is frozen at low temperature, has glass-like structure. Such ice offers to the high speed knife similar slicing characteristics as paraffin does to ordinary slicing. To give one example the technique may be carried out by placing a small specimen in a cone or candle formed by a tapered metal tube filled with water. The whole is then quickly frozen by immersing it in liquid nitrogen or better in isopentane or propane, cooled to —170°C. by liquid nitrogen. After a few moments of freezing, the metal tube is removed and the frozen candle placed directly into the nitrogen cooled clamp of the feeding mechanism. The cutting wheel and the end of the feeding mechanism are enclosed in a chamber which is likewise cooled by liquid nitrogen.[6] Slicing then is carried out at tem-

[5] The Review of Scientific Instruments, vol. 17, no. 1, pp. 23–25, January, 1946.

[6] It is our experience that solid carbon dioxide ("Dry Ice") cannot be used for the cooling necessary in low temperature cutting. The reason is that it contains small quantities of non-volatile lubricating oil from the condensing operation and that this oil in the form of microscopical droplets gets into the circulating air current and is carried by it into the sections, destroying thereby much of the detail and contrast of the electron pictures.

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perature well below —30°C., to preserve the non-crystalline character of the ice. The heavier sections for light microscopic examination may be allowed to come to room temperature. The thin sections for electron microscopy require most careful low temperature freeze-drying for the removal of all water. A paper on this specific subject is in preparation.

DISCUSSION

It must be reasoned that only by the development of a suitable sectioning technique, could the vast experience gained in the interpretation of light microscope sections be applied to the examination of electron micrographs of sections. The immense advantage that the fulfillment of this would mean, can be understood even more thoroughly when it is remembered that at least 50 years have been spent in developing to the present state of near perfection, the study and interpretation of sections of biological material by the use of the light microscope. Yet the limitation of thickness makes the known techniques inadequate for the electron microscope, since the thinnest possible sections which can be produced by them are many times thicker (generally by a factor of 10) than the electron beam is able to penetrate. Highly valuable and extensive experience seems thereby excluded from application to the new instrument.

Neither of the two techniques, the one by segregation of cell parts and the other by growing thin cells, allows an examination of biological materials and cell structures as they naturally exist. The segregation method has found its value in the investigation of the smaller particulate parts of the cell. But cells must be fully disintegrated before segregation can be obtained and all investigation of structure and spatial arrangement is consequently impossible. Particles as large as the nuclei, are in themselves too large to allow sufficient electron penetration to produce detail.

The "thin-grown" cell method presupposes specimens of living tissue and it is thereby limited to a comparatively small field of investigation in which living tissue is available,

as well as by the time it takes to grow the thin cells. Tissue as it normally exists cannot be investigated by this technique. Furthermore, it must still be proven that growth taking place preponderably in two dimensions does not produce a serious distortion of the cells. These may be the reasons that the method which has been known for many years has found only a limited field in light microscopy.

We believe that the high speed microtome, making possible sections of the required thinness, offers a solution of the problem. Our experiments seem to indicate that its role in electron microscopy will be in direct parallel to the role of the ordinary microtome in light microscopy. Figures 4 to 8 show this parallel clearly in the similarity of structure. Furthermore they illustrate the advantage of the electron microscope due to its superior resolution and higher magnification.

The electron microscope would attain a position of prominence if it could do no more than offer increased resolution and magnification in the study of biological material. But another point is certain to strengthen its position still more. It has the possibility of producing three dimensional micrographs. The light microscope is not capable of doing this because of its shallow depth of field. Electron micrographs of sections can be produced not only at a resolution and magnification far superior to light micrographs, but they also show the three dimensional, structural arrangement existing within the tissue. Figures 10 to 12 indicate that the fundamentals for such a major widening of the extent of microscopical study exist and can be used. Before they are fully perfected, however, a number of auxiliary techniques will have to be worked out. Tissue fixation and staining must be experimented with further.

The reason that most fixatives used in light microscopical techniques are unsuitable arises from the fact that specimens must be as free from volatile substances as possible before they are exposed to the vacuum in the electron microscope. An electron beam can only be maintained in high vacuum. Tissue in the average contains 10 to 25% dry matter, the rest is water — most of it in colloidal combination with proteins.

The evaporation of 75 to 90% of the weight of a tissue is apt to produce severe shrinkage unless a meticulous technique is employed to prevent artifacts. Freeze-drying may solve this problem, though our extended work with it has as yet not produced fully satisfactory results. The well known fixatives, like formalin or Zenker's solution yielded pictures in which the non-particulate parts of the cell are seriously distorted. This is illustrated in figures 9 and 10. Fixation by osmium tetroxide we found to be more satisfactory. It does not seem to coagulate the semi-fluid parts of the cell but rather exerts a general over-all staining action on all parts of the tissue, browning or blackening it. Osmium tetroxide is a strong oxidizing agent and its action is based on oxidation of organic material such as proteins and fats. The reaction is particularly pronounced with fats, either finely dispersed through the tissue or in globule form, and it is most pronounced with unsaturated fats. As osmium tetroxide gives off oxygen it is reduced to a lower insoluble osmium oxide, which on account of the high atomic mass of osmium is highly opaque to an electron beam. The more osmium present in a certain tissue part, the denser that part will appear in an electron graph. It acts thereby as an overall stain which possesses in addition some selectivity towards fats. For this reason the fat-containing parts of tissue appear darker in our electron micrographs. Mitochondria, for instance, containing considerable amounts of unsaturated fats appear particularly pronounced. Figures 5 to 8, 11 and 12 illustrate some of these qualities of osmium tetroxide.

The method of slicing with ice as embedding material we believe opens new possibilities in even other fields than tissue study. We have reason to believe that the new technique may be valuable in bacteriology. Bacterial cultures, concentrated by centrifugation, can, for instance, be frozen in candle form and sliced by the high speed microtome, yielding numerous sections, cut at all different angles and through all different parts of the bacteria. This may allow a more thorough study of the morphology of bacteria than has been possible so far,

and may throw new light on a number of unsolved questions.[7] The particulate enclosures of bacteria or the thickness and structure of their enveloping membranes, the latter apparently playing an important part in combating mycobacterial diseases like tuberculosis and leprosy, may be studied in a detail not heretofore possible. It can be expected that segregated cell constituents, like nuclei, mitochondria, chromatin threads, the Golgi apparatus, etc., may be similarly sectioned and studied.

We hope that the ice embedding technique may, for example, allow the study of some phases of cell mitosis. Concentrated segregates of nuclei of fast growing tissue like regenerating liver or embryonic or cancerous tissue may be sectioned with this new technique. It can be expected that the sections will display numerous examples of different stages of mitosis and will reveal the chromosome formation present at the moment when the process was arrested by the specimen preparation.

The color stains used in light microscopy do not promise much for the electron microscope. Since the instrument deals with an electron beam instead of a light beam and since no color as such is revealed by electrons, color stains can offer only the one hope that some of them might increase selectively the opacity of tissue structures towards electrons.

SUMMARY

High speed microtome sectioning of biological material is described. It is possible to produce sections a fraction of a micron in thickness. These are thin enough to be used in the electron microscope. Sections of the usual thicknesses for the light microscope can also be obtained. Methods of tissue fixation and embedding are given.

The use of a new class of embedding materials which volatilize by sublimation and which eliminate the drawback of extraction by solvents is described. The novel possibility of

[7] A paper is in preparation on bacteria sectioning.

using ice for embedding and slicing purposes may open some new phases of microscopical research.

A number of micrographs are given which illustrate the successful technique of high speed microtome sectioning as well as the necessary auxilliary techniques, particularly fixation, embedding and section collecting. The figures indicate some of the fields of application of the new microtome.

Special thanks are expressed to Dr. Albert Claude of the Rockefeller Institute for Medical Research, who prepared and furnished many of the tissue samples and offered valuable advice and also to Mary C. Schuster of the Research Laboratories of Interchemical Corporation, who suggested and developed the described volatile embedding materials. The value of the assistance of Clifford Gray, Olive Hodgson, Arthur Koch, Gertrude Pfeiffer and Vincent Salines, all members of the Research Laboratories of Interchemical Corporation, is gratefully acknowledged.

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EXPLANATION OF PLATES

The micrographs accompanying this paper are given with the sole object of illustrating that tissue sections suitable for electron microscopic examination can be made by the high speed microtome. The tissue selected for this work was mostly liver tissue. It was reasoned that if this delicate tissue with its many inclusions could be sliced satisfactorily it could be safely concluded that almost any other tissue could also be sliced satisfactorily. A few tumor sections are also presented.

No attempt is made to offer at this time a cytological study of the sections and only passing mention is made of some cell parts which we believe are unmistakably presented in the pictures.

PLATE 1

EXPLANATION OF FIGURE

1 An over-all view of our high speed microtome. The speeds of the knife bearing wheel and the speeds of the sample forwarding mechanism are controlled by the board in the foreground. The stroboscope on top of the control board is used for checking motor and knife speeds. The microtome occupying the background of the picture consists of the feeding mechanism of the sample on the left, the motor and spindle for the knife bearing wheel in the center, and the section collecting apparatus on the right. It is mounted on a heavy steel support over which fits a heavy steel and plate glass cover (not shown in picture) for the protection of the observer. The base is also equipped for a heavy glass vacuum bell (not shown) in case working in vacuum is desirable in order to lessen loss of sections by air turbulence.

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PLATE 1

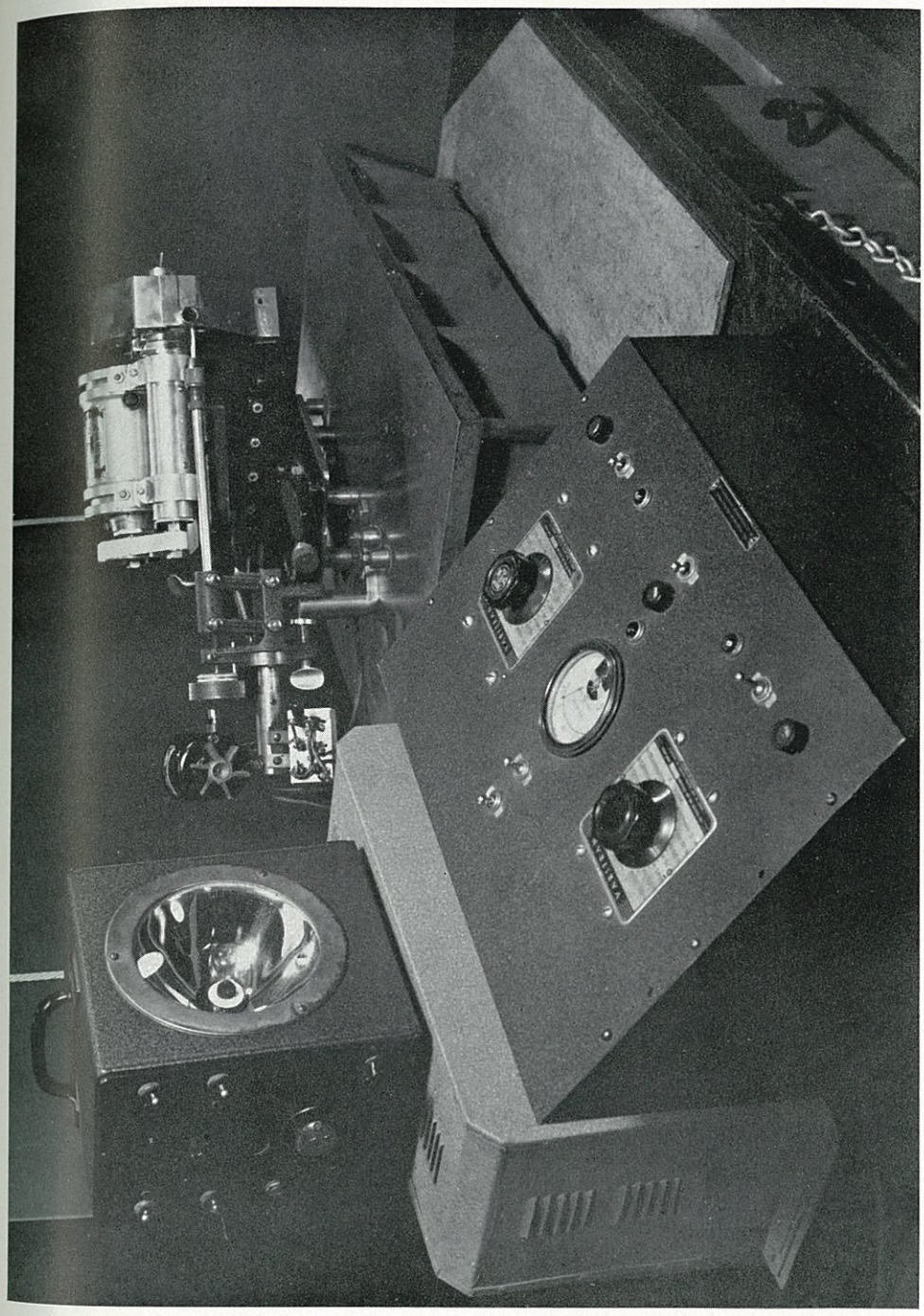

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PLATE 2

EXPLANATION OF FIGURE

2 This shows in more detail the driving motor and knife bearing wheel with most of the sample collecting apparatus removed to obtain this view. The sample forwarding mechanism is in the foreground.

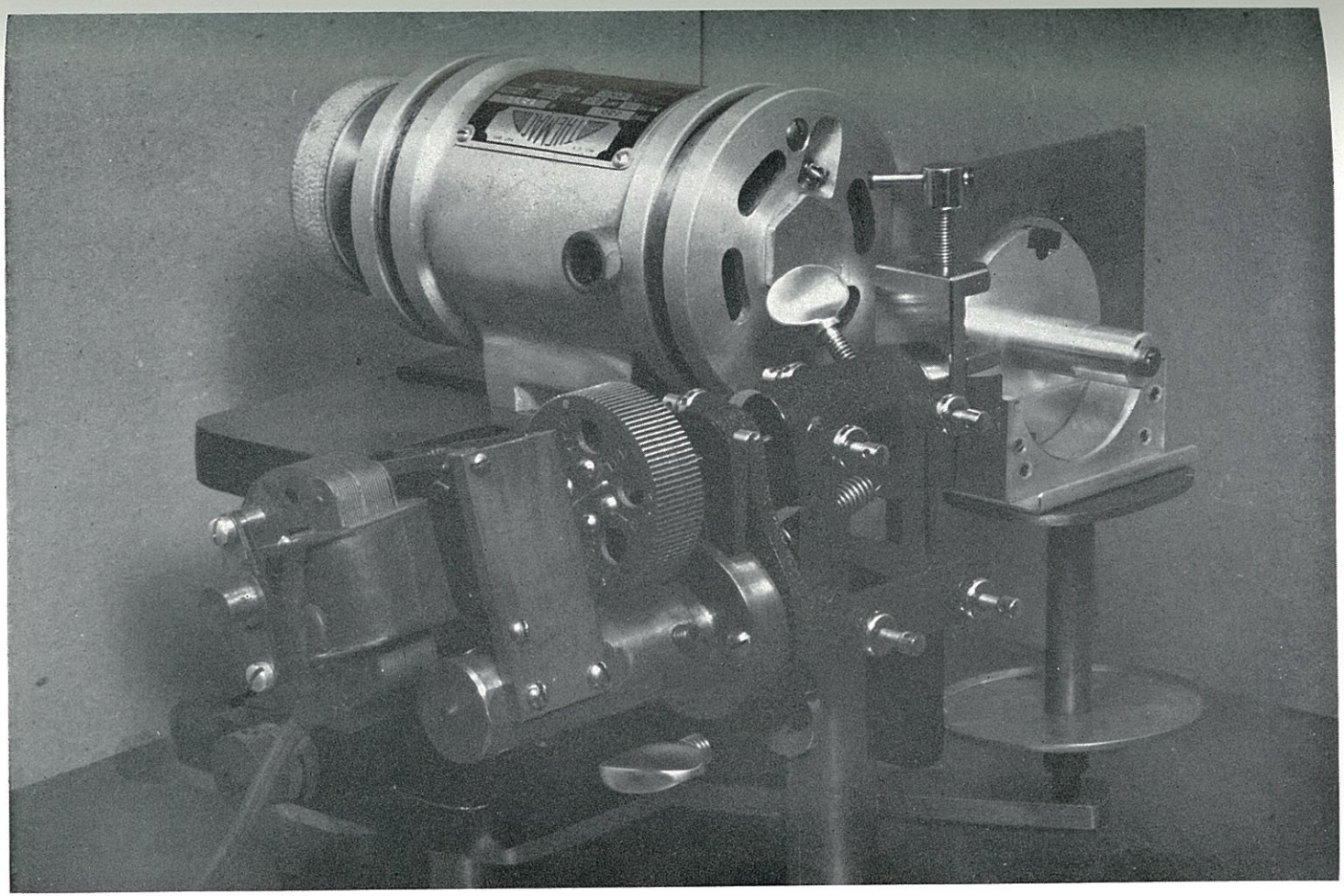

PLATE 3

EXPLANATION OF FIGURE

3 A close-up of the knife bearing wheel with its driving spindle and motor back of it. The slicing knife is seen on the left side of the wheel above the specimen to be sliced which is held by a drill chuck. Again, most of the sample collecting apparatus has been removed to obtain the view.

PLATE 3

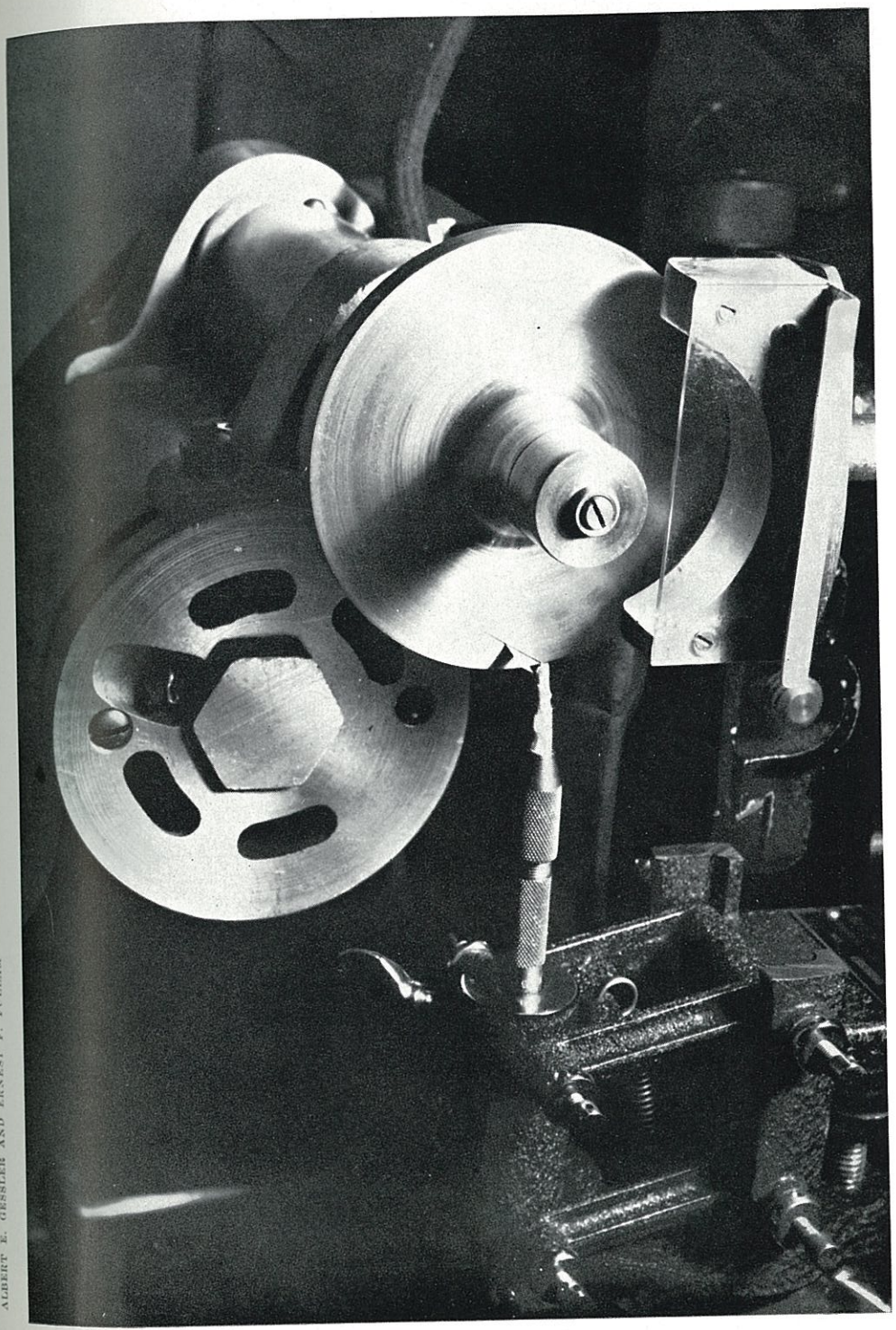

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PLATE 4

EXPLANATION OF FIGURE

4 A light micrograph of the thinnest section obtainable on a high grade microtome (Spencer Sliding Microtome). It is a section of pig's liver of an average thickness of 1 micron and is stained with hematoxylin. Some of the nuclei show that they have been sectioned. Mitochondria and cell walls are visible, but rather indistinctly. Taken at 1400 ×, enlarged to 2200 ×.

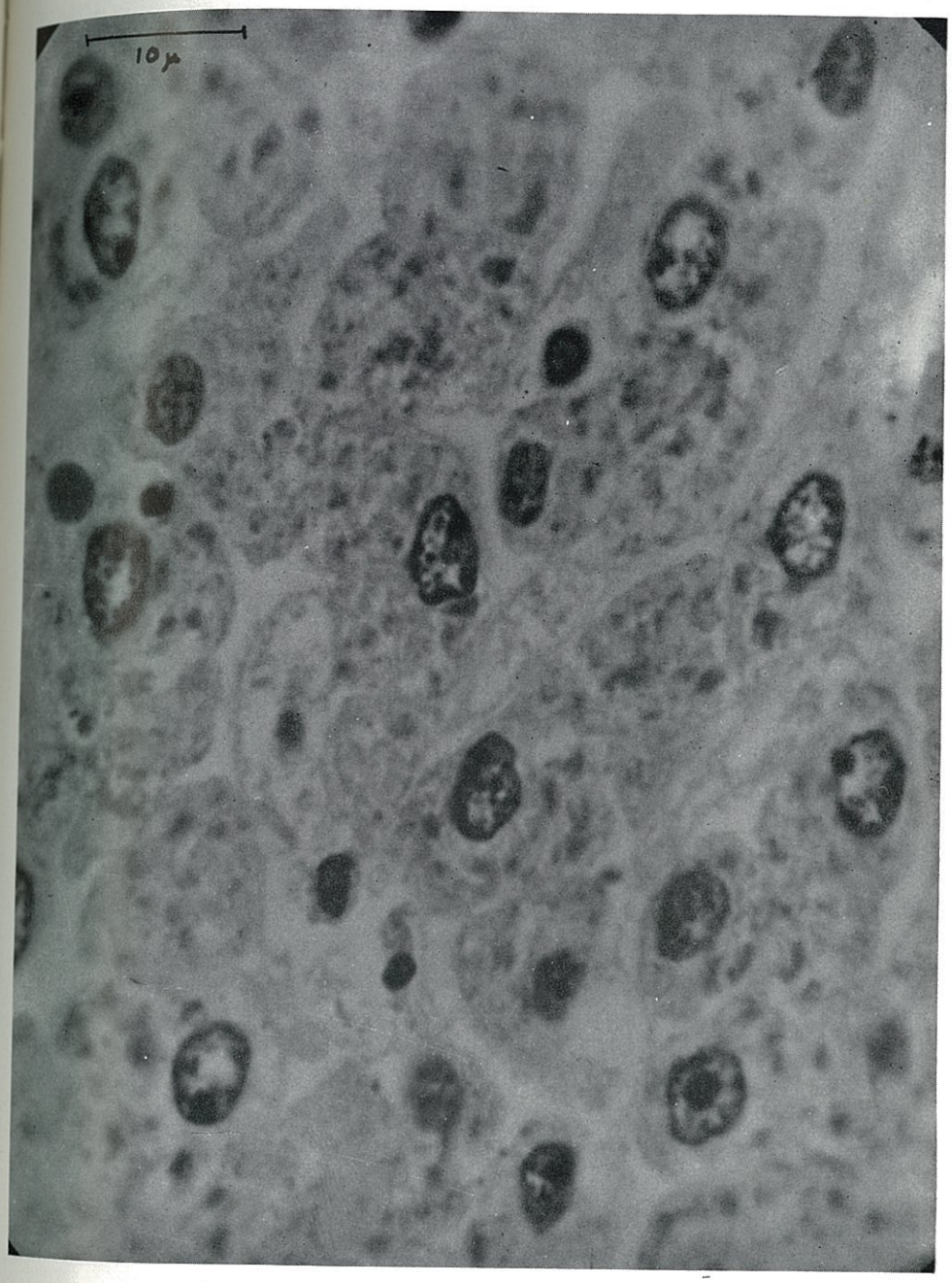

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PLATE 5

EXPLANATION OF FIGURE

5 An electron micrograph of guinea pig liver sliced by the high speed microtome to ca. 0.2 micron thickness. The specimen was previously fixed with osmium tetroxide and embedded in paraffin. Satisfactory electron penetration is demonstrated by clear cut cell walls of sharp contrast and by fair detail of cytoplasmic structure and mitochondria. The sections of nuclei show fine nuclear material and some of the nucleoli appear to have been hit by the knife and partly moved from their original position, thereby indicating a denser structure than that of the other parts of the nucleus. The sharply bounded white spaces between the cells we believe to be bile capillaries and lymph spaces. Taken at 1650 ×, enlarged to 4500 ×.

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PLATE 6

EXPLANATION OF FIGURE

6 A higher electron magnification, prepared like figure 5, shows a high speed microtome section with cell walls, ducts, mitochondria and nuclei sharply reproduced. Some of the cytoplasmic material seems to indicate particularly delicate structure. Taken at 3000 ×, enlarged to 8000 ×.

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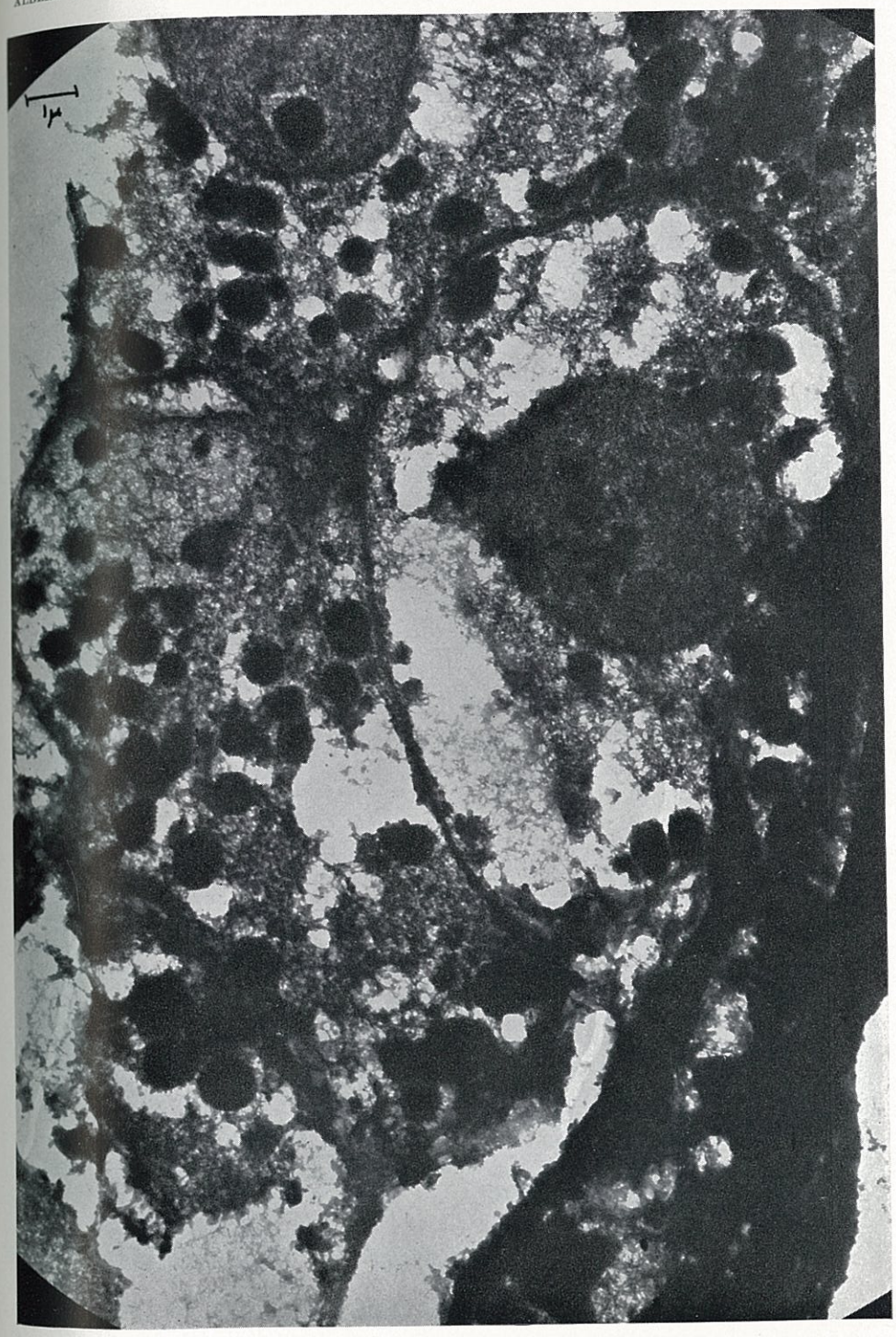

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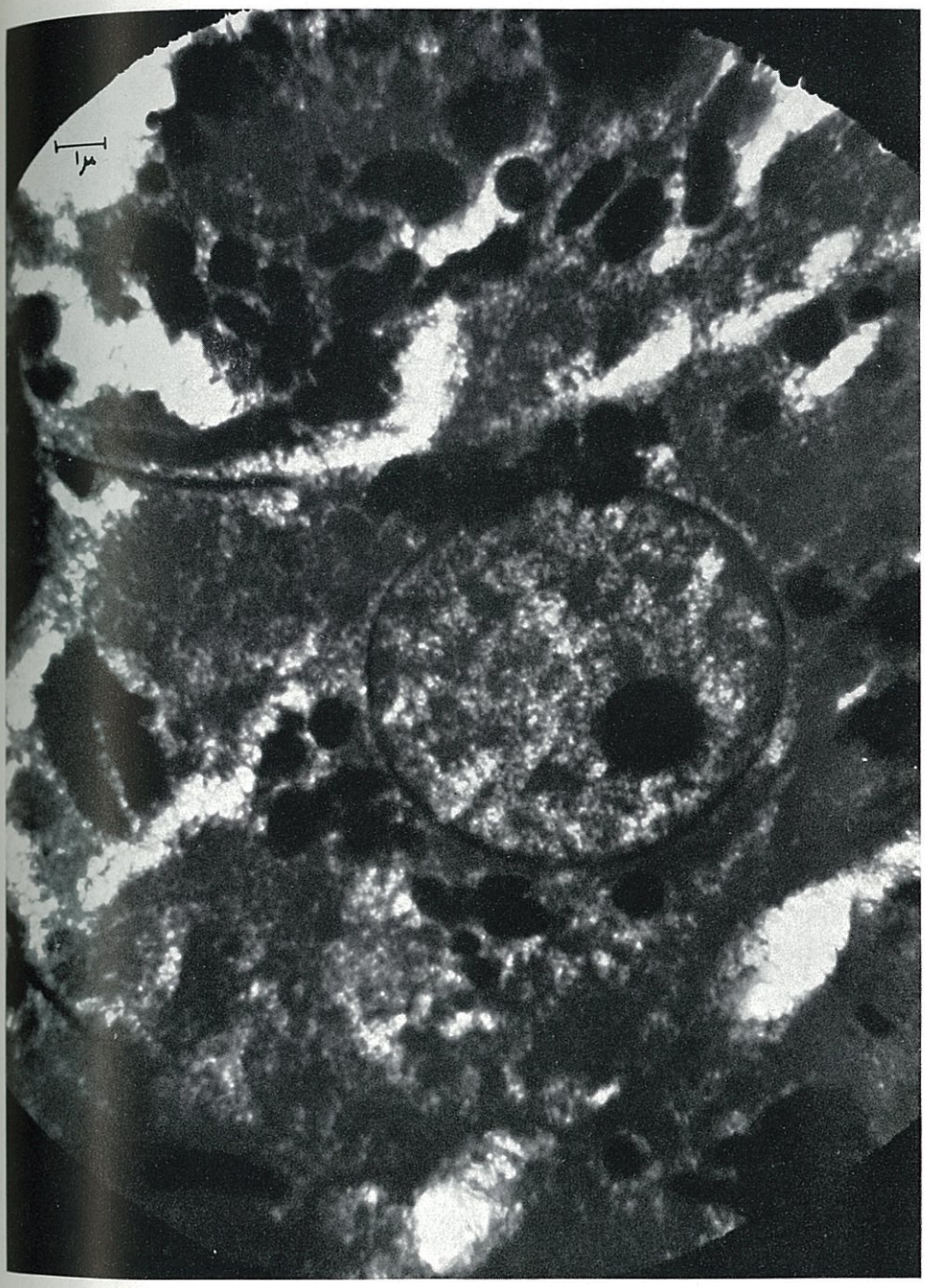
PLATE 7

EXPLANATION OF FIGURE

7 An electron micrograph of a high speed microtome section of guinea pig liver fixed in osmium tetroxide and embedded in paraffin. A nucleus sectioned in disk-form occupies the center of the picture and the nuclear membrane and nucleolus are sharply shown. Taken at 2700 ×, enlarged to 7000 ×.



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A nucleus sectioned in disk-
ar membrane and nucleolus
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PLATE 8

EXPLANATION OF FIGURE

8 This is similar to figure 6 but at still higher magnification. It features a sectioned nucleus in which one of the two nucleoli appears to be also sectioned. Taken at 5000 ×, enlarged to 13,000 ×.

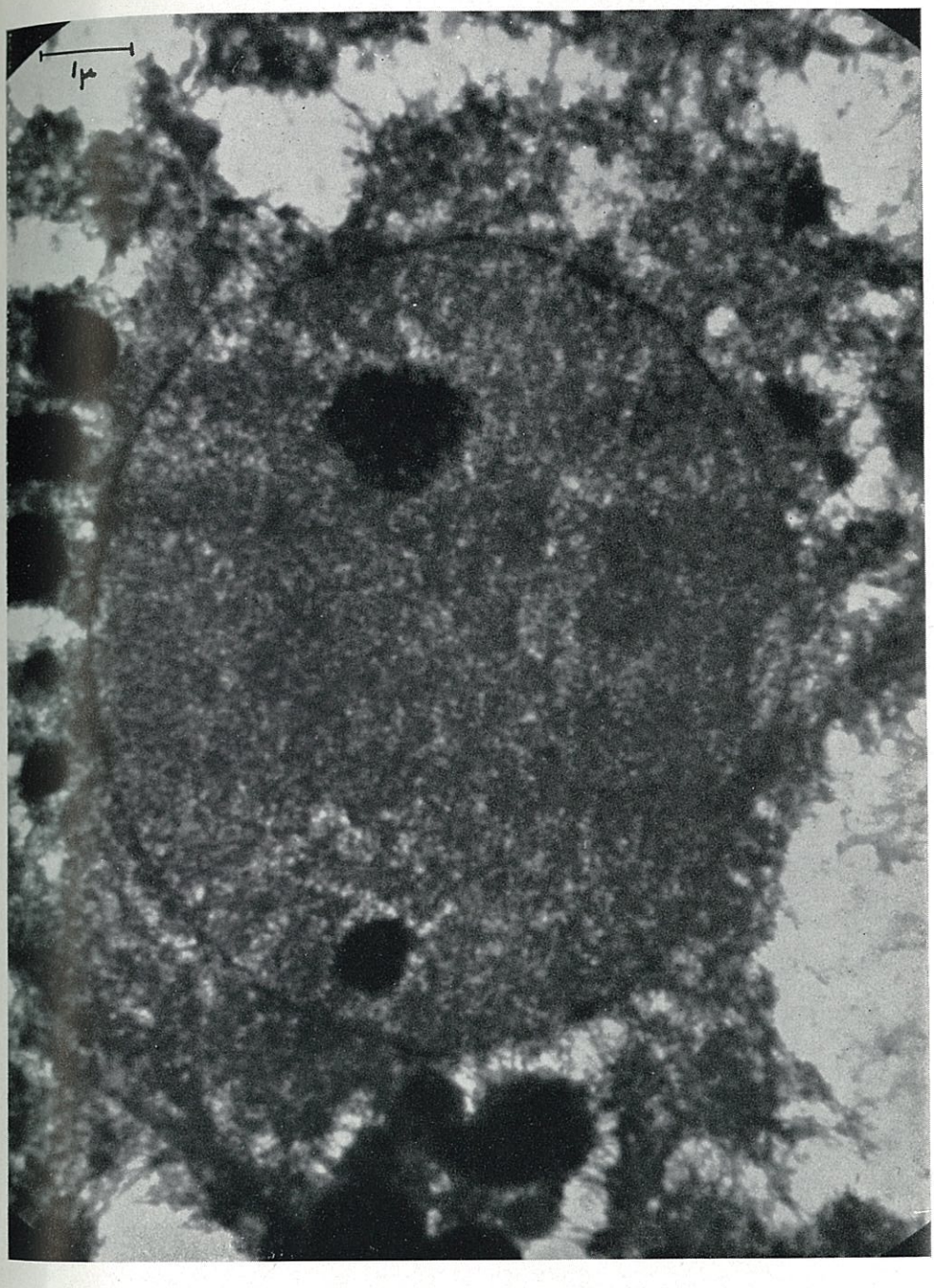

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PLATE 9

EXPLANATION OF FIGURE

9 An electron micrograph of a high speed microtome section of a rat tumor tissue fixed in 10% formalin. This is at a comparatively low magnification. While much detail was lost by this fixation, the picture is of interest, notwithstanding, since it seems to show with particular clearness the cell formation of the walls of a capillary. Many of the nuclei are of oblong shape. Taken at 1050 ×, enlarged to 4000 ×.

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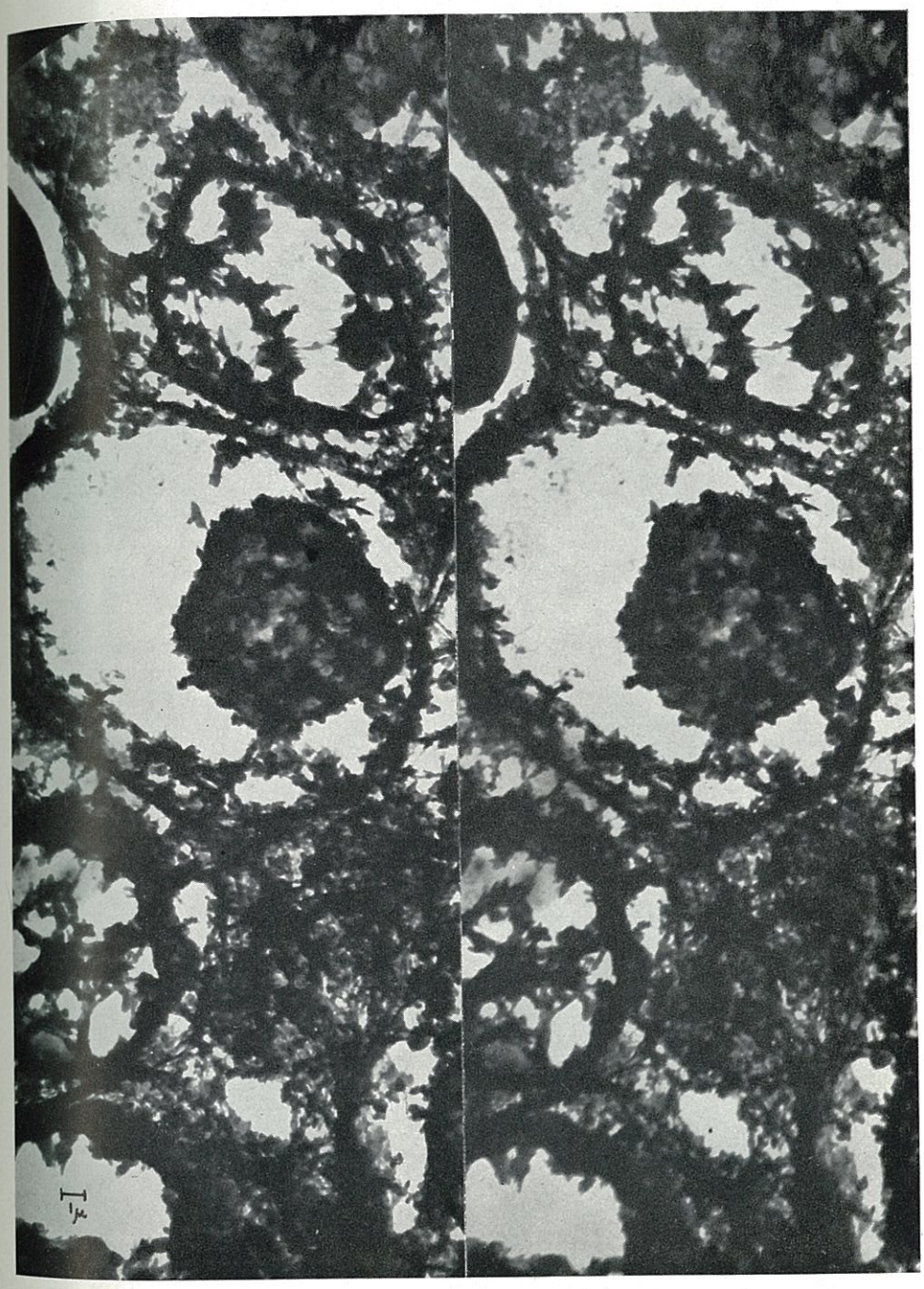
Taken at 1050 ×, en-

PLATE 10

EXPLANATION OF FIGURE

10 A stereoscopic electron micrograph of a high speed microtome section of transplanted rat leukemia tumor. For its preparation, Zenker's fixatives and paraffin embedding were used. The distortion and loss, particularly of the cytoplasmic structure, is apparent in contrast to the osmium tetroxide fixation of some of the other pictures. The distortion by the fixative, however, did not affect the clearly defined cell walls and nuclear membranes, but it destroyed most of the more delicate structures. The nucleus in the center, in this case, has been sectioned by the knife in a position to leave a cup-shaped section showing great three-dimensional depth if viewed through a stereoscope. Taken at 1250 ×, enlarged to 3000 ×.



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cope. Taken at 1250 ×, en-

PLATE 11

EXPLANATION OF FIGURE

11 A section of guinea pig liver thoroughly fixed with osmium tetroxide and embedded in paraffin, showing excellent retention of fine detail. It is fortunate in showing two nuclei in very close contact. Taken at 2700 ×, enlarged to 5000 ×.

12 An electron microscopic stereograph of a high speed microtome section of guinea pig liver fixed with osmium tetroxide and embedded in camphor naphthalene. The clarity this method is able to produce is shown in the details of the nuclear wall, the nucleoli and nuclear internal structure. Taken at 5000 ×, enlarged to 6500 ×

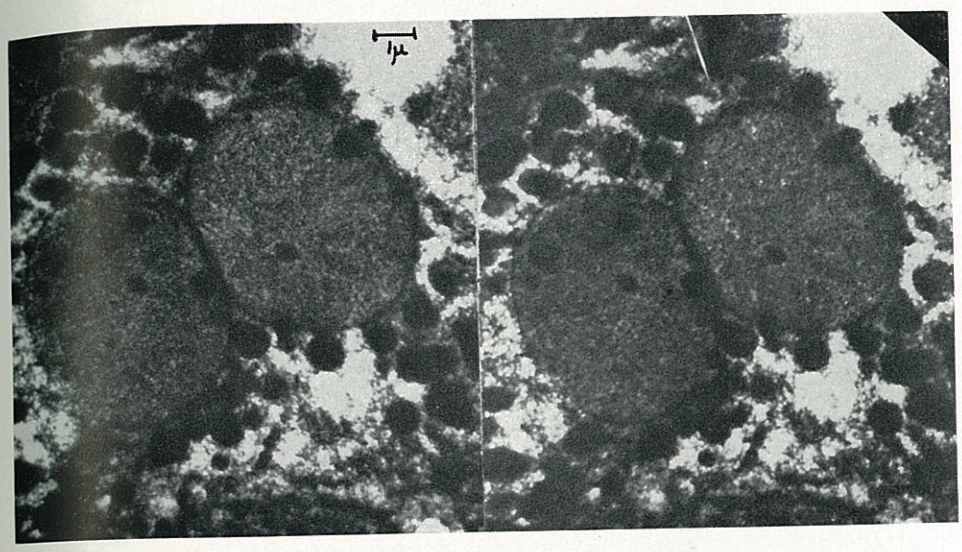

Figure 11

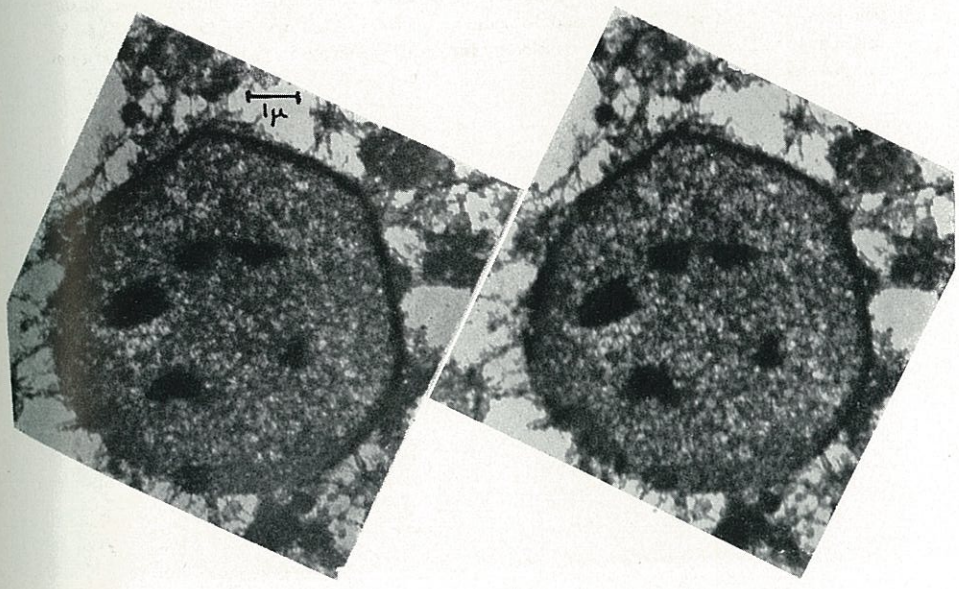

Figure 12

Extra figure of stereoscopic electron micrograph for cutting and mounting.

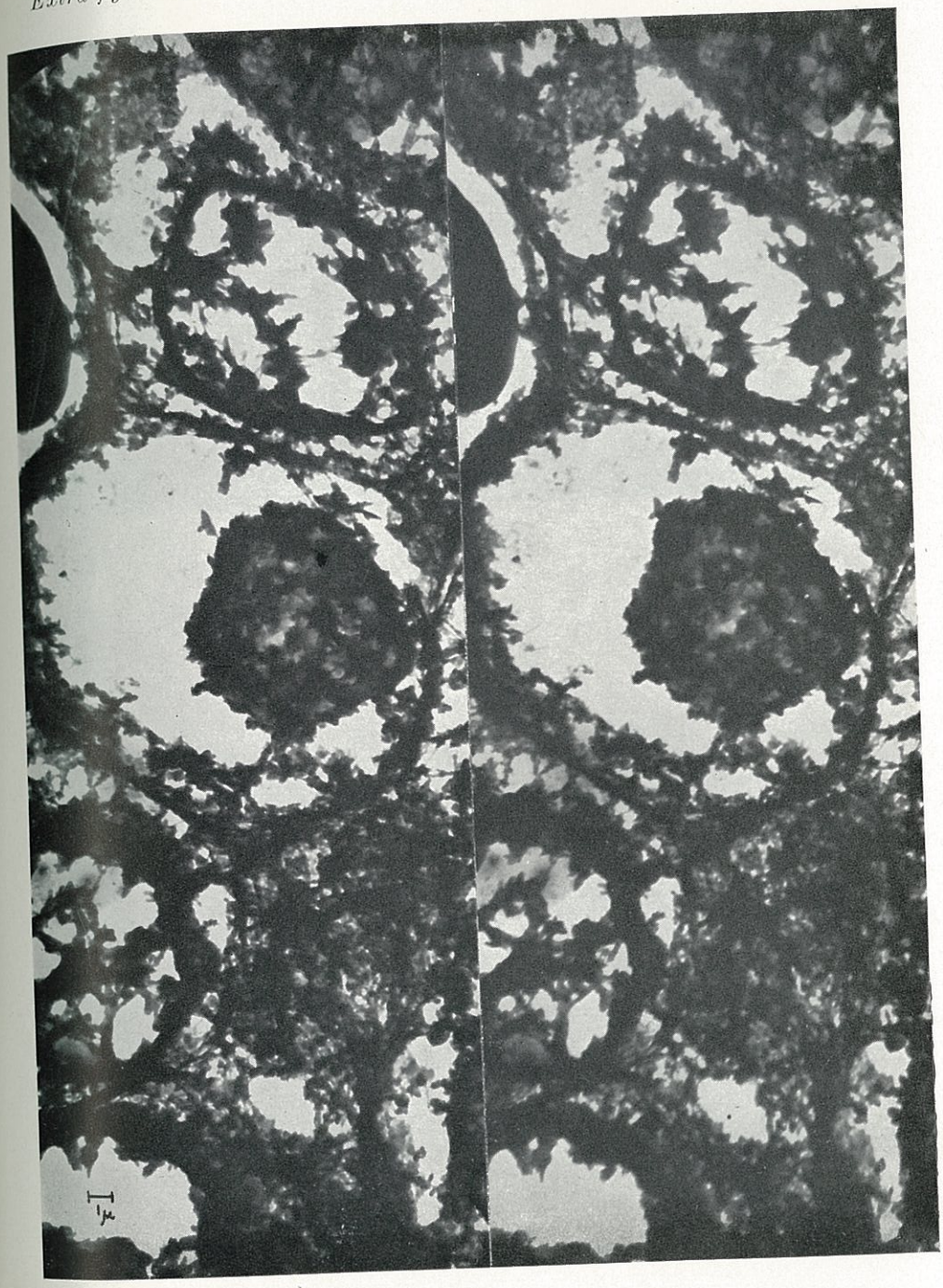

Figure 10

SECTIONING FOR ELECTRON MICROSCOPE

Albert E. Gessler and Ernest F. Fullam

Extra figure of stereoscopic electron micrograph for cutting and mounting.

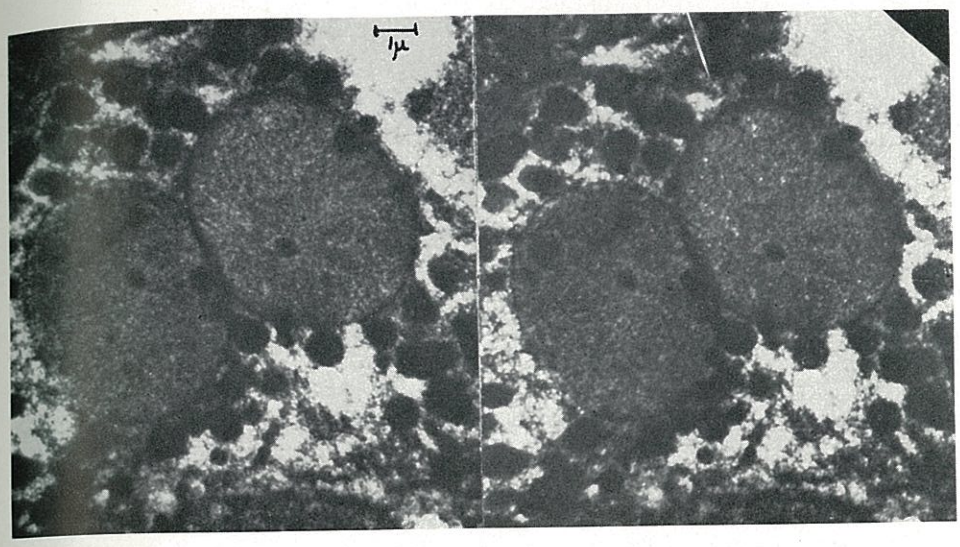

Figure 11

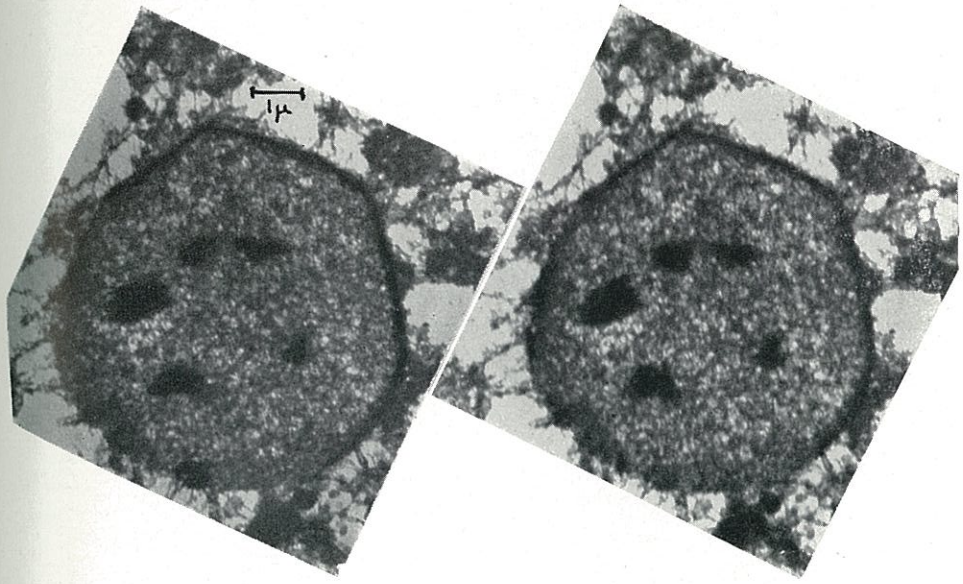

Figure 12

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